Now what...?

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Directed differentiation of human pluripotent stem cells into functional insulin-producing beta-like cells holds great promise for cell replacement therapy for patients suffering from diabetes. This approach also offers the unique opportunity to study otherwise inaccessible aspects of human beta cell development and function in vitro. Here, we show that current pancreatic progenitor differentiation protocols promote precocious endocrine commitment, ultimately resulting in the generation of non-functional polyhormonal cells. Omission of commonly used BMP inhibitors during pancreatic specification prevents precocious endocrine formation while treatment with retinoic acid followed by combined EGF/KGF efficiently generates both PDX1+ and subsequent PDX1+/NKX6.1+ pancreatic progenitor populations, respectively. Precise temporal activation of endocrine differentiation in PDX1+/NKX6.1+ progenitors produces glucose-responsive beta-like cells in vitro that exhibit key features of bona fide human beta cells, remain functional after short-term transplantation, and reduce blood glucose levels in diabetic mice. Thus, our simplified and scalable system accurately recapitulates key steps of human pancreas development and provides a fast and reproducible supply of functional human beta-like cells.

Controlled induction of human pancreatic progenitors produces functional beta-like cells in vitro

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Can we find ways to restore & replace pancreatic β-cells?

Diabetes type I and II

Transplantation of cadaveric islets works for a short amount of time.
Limited supply and poor donor quality

What the limitless potential of using human embryonic stem cells hESC?

Directed differentiation of hES cells into mature beta-like cells

Simplified diagram describing the new strategy reported for in vitro differentiation of hESCs into pancreatic beta-like cells. The addition of BMP inhibitors at pancreatic progenitor stage (PP) ensures precise temporal activation of NEUROG3 and favors generation of monohormonal insulin-secreting cells versus polyhormonal cells. Commonly used differentiation strategy is boxed in light gray, wherein earlier inhibition of BMP signaling leads to premature endocrine commitment and higher number of polyhormonal cells.

ORIGINAL ARTICLE

A free-choice high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity

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Metabolic Changes in Pregnancy

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A gestational ketogenic diet alters maternal metabolic status as well as offspring physiological growth and brain structure in the neonatal mouse

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Women of child bearing years

How does KD effect offspring

KD diet

Brain development?

Lipids and myelin synthesis?
During gestation – ketogenesis is limited

Ketone supply is from maternal circulation

Ketone supply is modulated by MCT transporters (monocarboxylate)

**KD dams**

↓ fertility
↓ litter size

↑ risk of developing fatal ketoacidosis by mid-lactation

Sussman *et al.* BMC Pregnancy and Childbirth 2013, 13:198
Conclusions:

• “A gestational ketogenic diet deleteriously affects maternal fertility and increases susceptibility to fatal ketoacidosis during lactation.

• Prenatal and early postnatal exposure to a ketogenic diet also results in significant alterations to neonatal brain structure, and results in retarded physiological growth.

• These alterations could be accompanied by functional and behavioural changes in later postnatal life.”
Maternal adaptations occur in multiple systems:
- cardiovascular,
- respiratory, and
- metabolic.

To compensate for these changes, both maternal hepatic gluconeogenesis and fatty acid levels increase.

In brief, during the first weeks of pregnancy, the presence of the fetal-placental unit causes a drop in growth hormone levels, resulting in enhanced insulin sensitivity (6). After this period of increased sensitivity to insulin, circulating levels of human placental lactogen, placenta-derived human growth hormone (GHI-V), progesterone, cortisol, prolactin, and other hormones increase and contribute to decreasing insulin sensitivity in peripheral tissues such as adipocytes and skeletal muscle by interfering with insulin receptor signaling (6). Elevated levels of these placenta- and non-placenta-derived hormones, particularly progesterone, cortisol, and GH-V, lead to markedly decreased insulin sensitivity during the second and third trimesters of pregnancy, with the highest levels of insulin resistance occurring during the third trimester (3). The role of placenta-derived hormones in mediating insulin resistance is made evident by the marked decrease in insulin resistance immediately postpartum (7).
Pregnancy is characterized by a progressive increase in nutrient-stimulated insulin responses despite an only minor deterioration in glucose tolerance... consistent with progressive insulin resistance.

In normal pregnant women, basal endogenous hepatic glucose production increased by 16–30% to meet the increasing needs of the placenta and fetus.

Metabolic adaptations do not fully compensate in GDM and glucose intolerance ensues.

GDM may reflect a predisposition to type 2 diabetes or may be an extreme manifestation of metabolic alterations that normally occur in pregnancy.

CARBOHYDRATE METABOLISM DURING NORMAL PREGNANCY

Increased insulin secretion and decreased insulin sensitivity

During early pregnancy, glucose tolerance is normal or slightly improved and peripheral (muscle) sensitivity to insulin and hepatic basal glucose production is normal (1–3). The hyperinsulinemic-euglycemic glucose clamp technique and computer-assisted intravenous-glucose-tolerance test indicate greater-than-normal sensitivity to the blood glucose-lowering effect of exogenously administered insulin in the first trimester than in the second and third trimesters. Insulin responses to oral glucose are also greater in the first trimester than before pregnancy. These observations are consistent with a 120% increase at 12–14 wk gestation in the first phase of insulin response, which refers to the change in insulin concentration relative to the elevation in glucose concentration from 0 to 5 min after intravenous glucose administration. The second phase of insulin response, which refers to the rate of insulin release relative to the glucose concentration 5 to 60 min after intravenous glucose administration, is not significantly different in early pregnancy from the pregravid state. The cause of the enhanced insulin secretion is uncertain because peripheral insulin sensitivity and hepatic glucose production rates are not different from pregravid values. This metabolic milieu under the influence of cortisol, estrogens, and progestins favors lipogenesis and fat storage.
Longitudinal studies of glucose tolerance during gestation show a progressive increase in nutrient-stimulated insulin responses despite an only minor deterioration in glucose tolerance, consistent with progressive insulin resistance (4). The hyperinsulinemic-euglycemic glucose clamp technique and computer-assisted intravenous-glucose-tolerance test indicate that insulin action in late normal pregnancy is 50–70% lower than that of normal, nonpregnant women (1–3, 5, 6). A progressive increase in basal and postprandial insulin concentrations is seen with advancing pregnancy. By the third trimester, basal and 24-h mean insulin concentrations may double (7). The first and second phases of insulin release are 3- to 3.5-fold greater in late pregnancy (1). Obese pregnant women also develop peripheral and hepatic insulin resistance during the third trimester of pregnancy (8). The hyperinsulinemic-euglycemic glucose clamp technique indicates that insulin-stimulated glucose disappearance, carbohydrate oxidation, and suppression of endogenous glucose production in obese women are reduced in the third compared with the second trimester.

Although the precise mechanism is uncertain, alterations in the hormonal milieu during pregnancy are probably responsible for the reduced insulin sensitivity. Changes in β cell responsiveness occur in parallel with growth of the fetal placental unit and its elaboration of hormones such as human chorionic somatomammotropin (HCS), progesterone, cortisol, and prolactin. Prevailing insulin resistance produces exaggerated changes in postprandial concentrations of metabolic fuels (eg, glucose, VLDL, and amino acids). Insulin resistance serves to shunt ingested nutrients to the fetus after feeding.
Gestational diabetes mellitus

GDM is defined as “carbohydrate intolerance of variable severity with onset or first recognition during the present pregnancy” (17). GDM is a heterogeneous disorder in which age, obesity, and genetic background contribute to the severity of the disease. Women with GDM are at risk for later development of type 2 diabetes. Only a 1.6% incidence of islet cell antibodies are found by using a specific monoclonal antibody in women with GDM (18). GDM is accompanied by alterations in fasting, postprandial, and integrated 24-h plasma concentrations of amino acids, glucose, and lipids. These changes include a 3-fold increase in plasma triacylglycerol concentrations during the third trimester of pregnancy, elevation of plasma fatty acids, delayed postprandial clearance of fatty acids, and elevation of the branched-chain amino acids (19).

The pathophysiology of GDM remains controversial; GDM may reflect a predisposition to type 2 diabetes expressed under the metabolic conditions of pregnancy or it may represent the extreme manifestation of metabolic alterations that normally occur in pregnancy. GDM is not due to defective secretion of insulin or to disproportionate secretion of proinsulin or glucagon (4). Only quantitative differences in insulin secretion have been observed between women with GDM and normal pregnant women. Evidence supports the view that GDM is related to a pronounced peripheral resistance to insulin.
Hyperglycemia accentuates and ketonemia attenuates hypoglycemia-induced neuronal injury in the developing rat brain

Kathleen Ernis, Hannah Dotterman, Ariel Stein and Raghavendra Rao

METHODS: Three-week-old rats were subjected to insulin-induced hypoglycemia and treated with 10% dextrose or 50% dextrose. Neuronal injury, PARP-1, and brain-derived neurotrophic factor (BDNF) III/TrkB/p75NTR expressions were determined. In the second experiment, ketonemia was induced by administering β-hydroxybutyrate during hypoglycemia and its effect on neuronal injury was compared with those conventionally treated using 10% dextrose.

RESULTS: Both 10 and 50% dextrose administration led to hyperglycemia (50% dextrose > 10% dextrose). Compared with the 10% dextrose group, neuronal injury was greater in the 50% dextrose group and was accompanied by PARP-1 overactivation. BDNF III and p75NTR, but not TrkB, mRNA expressions were upregulated. Neuronal injury was less severe in the rats subjected to ketonemia, compared with those conventionally treated using 10% dextrose.

BACKGROUND: Prolonged hypoglycemia leads to brain injury, despite treatment with 10% dextrose. Whether induction of hyperglycemia or ketonemia achieves better neuroprotection is unknown. Hyperglycemia is neuroprotective in other brain injuries during development; however, it worsens hypoglycemia-induced injury in the adult brain via poly(ADP-ribose)polymerase-1 (PARP-1) overactivation.

CONCLUSION: Hyperglycemia accentuated hypoglycemia-induced neuronal injury, likely via PARP-1 overactivation. Although BDNF was upregulated, it was not neuroprotective and potentially exaggerated injury by binding to p75NTR receptor. Conversely, ketonemia during hypoglycemia attenuated neuronal injury.
Self-reported short sleep duration and frequent snoring in pregnancy: impact on glucose metabolism

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OBJECTIVE: We sought to evaluate the impact of short sleep duration (SSD) and frequent snoring (FS) on glucose metabolism during pregnancy.

STUDY DESIGN: We conducted a prospective cohort study of healthy nulliparas who participated in a sleep survey study. SSD was defined as <7 hours of sleep per night and FS, as snoring ≥3 nights per week. Outcomes included 1-hour oral glucose tolerance results and the presence of gestational diabetes mellitus (GDM). Univariate and multivariate analyses were performed.

RESULTS: A total of 189 women participated; 48% reported an SSD and 18.5% reported FS. SSD and FS were associated with higher oral glucose tolerance values: SSD (116 ± 31 vs 105 ± 23; \(P = .008\)) and FS (118 ± 34 vs 108 ± 25; \(P = .04\)). Both SSD (10.2% vs 1.1%; \(P = .008\)) and FS (14.3% vs 3.3%; \(P = .009\)) were associated with a higher incidence of GDM. Even after controlling for potential confounders, SSD and FS remained associated with GDM.

CONCLUSION: SSD and FS are associated with glucose intolerance in pregnancy.

Key words: gestational diabetes, glucose metabolism, sleep disorders in pregnancy

The \( \beta \)-hydroxybutyrate receptor HCA\(_2\) activates a neuroprotective subset of macrophages

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The ketone body β-hydroxybutyrate (BHB) is an endogenous factor protecting against stroke and neurodegenerative diseases, but its mode of action is unclear. Here we show in a stroke model that the hydroxy-carboxylic acid receptor 2 (HCA₂, GPR109A) is required for the neuroprotective effect of BHB and a ketogenic diet, as this effect is lost in Hca2⁻⁻ mice. We further demonstrate that nicotinic acid, a clinically used HCA₂ agonist, reduces infarct size via a HCA₂-mediated mechanism, and that noninflammatory Ly-6C<sup>Lo</sup> monocytes and/or macrophages infiltrating the ischemic brain also express HCA₂. Using cell ablation and chimeric mice, we demonstrate that HCA₂ on monocytes and/or macrophages is required for the protective effect of nicotinic acid. The activation of HCA₂ induces a neuroprotective phenotype of monocytes and/or macrophages that depends on PGD<sub>2</sub> production by COX1 and the haematopoietic PGD<sub>2</sub> synthase. Our data suggest that HCA₂ activation by dietary or pharmacological means instructs Ly-6C<sup>Lo</sup> monocytes and/or macrophages to deliver a neuroprotective signal to the brain.
The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated inflammatory disease

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The ketone bodies β-hydroxybutyrate (BHB) and acetoacetate (AcAc) support mammalian survival during states of energy deficit by serving as alternative sources of ATP\(^1\). BHB levels are elevated by starvation, caloric restriction, high-intensity exercise, or the low-carbohydrate ketogenic diet\(^2\). Prolonged fasting reduces inflammation; however, the impact that ketones and other alternative metabolic fuels produced during energy deficits have on the innate immune response is unknown\(^3-6\). We report that BHB, but neither AcAc nor the structurally related short-chain fatty acids butyrate and acetate, suppresses activation of the NLRP3 inflammasome in response to urate crystals, ATP and lipotoxic fatty acids. BHB did not inhibit caspase-1 activation in response to pathogens that activate the NLR family, CARD domain containing 4 (NLRC4) or absent in melanoma 2 (AIM2) inflammasome and did not affect non-canonical caspase-11, inflammasome activation. Mechanistically, BHB inhibits the NLRP3 inflammasome by preventing K\(^+\) efflux and reducing ASC oligomerization and speck formation. The inhibitory effects of BHB on NLRP3 are not dependent on chirality or starvation-regulated mechanisms like AMP-activated protein kinase (AMPK), reactive oxygen species (ROS), autophagy or glycolytic inhibition. BHB blocks the NLRP3 inflammasome without undergoing oxidation in the TCA cycle, and independently of uncoupling protein-2 (UCP2), sirtuin-2 (SIRT2), the G protein–coupled receptor GPR109A or hydrocaboxylic acid receptor 2 (HCA2). BHB reduces NLRP3 inflammasome–mediated interleukin (IL)-1β and IL-18 production in human monocytes. In vivo, BHB or a ketogenic diet attenuates caspase-1 activation and IL-1β secretion in mouse models of NLRP3-mediated diseases such as Muckle–Wells syndrome, familial cold autoinflammatory syndrome and urate crystal–induced peritonitis. Our findings suggest that the anti-inflammatory effects of caloric restriction or ketogenic diets may be linked to BHB-mediated inhibition of the NLRP3 inflammasome.
The brain is dependent on glucose as a primary energy substrate, but is capable of utilizing ketones such as \( \beta \)-hydroxybutyrate and acetoacetate, as occurs with fasting, starvation, or chronic feeding of a ketogenic diet. The relationship between changes in cerebral metabolic rates of glucose (CMR\textsubscript{glc}) and degree or duration of ketosis remains uncertain. To investigate if CMR\textsubscript{glc} decreases with chronic ketosis, 2-[\textsuperscript{18}F]fluoro-2-deoxy-d-glucose in combination with positron emission tomography, was applied in anesthetized young adult rats fed 3 weeks of either standard or ketogenic diets. Cerebral metabolic rates of glucose (\( \mu \)mol/min per 100 g) was determined in the cerebral cortex and cerebellum using Gjedde–Patlak analysis. The average CMR\textsubscript{glc} significantly decreased in the cerebral cortex (23.0 ± 4.9 versus 32.9 ± 4.7) and cerebellum (29.3 ± 8.6 versus 41.2 ± 6.4) with increased plasma ketone bodies in the ketotic rats compared with standard diet group. The reduction of CMR\textsubscript{glc} in both brain regions correlates linearly by ~9% for each 1 mmol/L increase of total plasma ketone bodies (0.3 to 6.3 mmol/L). Together with our meta-analysis, these data revealed that the degree and duration of ketosis has a major role in determining the corresponding change in CMR\textsubscript{glc} with ketosis.
Sugar and Alzheimer’s disease: a bittersweet truth

Costantino Iadecola

Reductions in brain glucose metabolism have long been associated with Alzheimer’s disease. A study now demonstrates that the endothelial glucose transporter GLUT1 is vital for maintaining brain energy metabolism and vascular clearance of amyloid-β.
A class of molecules called carbohydrates.
HOW SUGAR AFFECTS THE BRAIN.